

CLAIMS

What is claimed:

1. An isolated DNA sequence encoding a polypeptide or fragment thereof, involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, said polypeptide comprising:

an amino acid sequence selected from the group consisting of SEQ ID NO: 1B, SEQ ID NO: 2B, SEQ ID NO: 3B, SEQ ID NO: 4B, SEQ ID NO: 5B, SEQ ID NO: 6B, an amino acid sequence having at least 30% homology with SEQ ID NO: 1B, amino acid sequence having at least 40% homology with SEQ ID NO: 2B, an amino acid sequence having at least 80% homology with SEQ ID NO: 3B, an amino acid sequence having at least 90% homology with SEQ ID NO: 4B, an amino acid sequence having at least 90% homology with SEQ ID NO: 5B, an amino acid sequence having at least 75% homology with SEQ ID NO: 6B, and a fragment of any thereof, said polypeptide or fragment thereof, being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

2. The isolated DNA sequence encoding a polypeptide or fragment thereof, of claim 1, wherein said amino acid sequence comprises at least 50% homology with SEQ ID NO: 1B, SEQ ID NO: 2B or a fragment of either thereof.

3. The isolated DNA sequence encoding a polypeptide or fragment thereof, of claim 1, wherein said amino acid sequence comprises at least 70% homology with SEQ ID NO: 1B, SEQ ID NO: 2B or a fragment of either thereof.

4. The isolated DNA sequence encoding a polypeptide, of claim 2, wherein said polypeptide or fragment thereof, has alcohol acyl transferase activity.

5. The isolated DNA sequence encoding a polypeptide, of claim 1, wherein said polypeptide or fragment thereof comprises SEQ ID NO: 3B and has aminotransferase activity.

6. The isolated DNA sequence encoding a polypeptide, of claim 1, wherein said polypeptide or fragment thereof comprises SEQ ID NO: 4B and has thiolase activity.

7. The isolated DNA sequence encoding a polypeptide, of claim 1, wherein said polypeptide or fragment thereof comprises SEQ ID NO: 5B and has pyruvate decarboxylase activity.

8. The isolated DNA sequence encoding a polypeptide, of claim 1, wherein said polypeptide or fragment thereof comprises SEQ ID NO: 6B and has alcohol dehydrogenate activity.

9. An isolated DNA sequence encoding a polypeptide involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, said isolated DNA sequence comprising:

a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1A, SEQ ID NO: 2A, SEQ ID NO: 3A, SEQ ID NO: 4A, SEQ ID NO: 5A, SEQ ID NO: 6A, SEQ ID NO: 7A, SEQ ID NO: 8A, SEQ ID NO: 9A, SEQ ID NO: 10A, SEQ ID NO: 11A, SEQ ID NO: 12A, SEQ ID NO: 13A, a nucleic acid sequence having at least 25% homology with SEQ ID NO: 1A, a nucleic acid sequence having at least 25% homology with SEQ ID NO: 2A, a nucleic acid sequence having at least 70% homology with SEQ ID NO: 3A, a nucleic acid sequence having at least 75 % homology to SEQ ID NO: 4A, a nucleic acid sequence having at least 75 % homology with SEQ ID NO: 5A, a nucleic acid sequence having at least 65% homology with SEQ ID NO: 6A, an amino acid sequence having at least 55% homology with one of SEQ ID NO: 7A, SEQ ID NO: 8A, SEQ ID NO: 9A or SEQ ID NO: 10A, a nucleic acid sequence having at least 48 % homology with SEQ ID NO: 11A, a nucleic acid sequence having at least 20% homology with SEQ ID NO: 12A or SEQ ID

NO: 13A, SEQ ID NO:15A, a nucleic acid sequence having at least 53% homology with SEQ ID NO:15A, and a complementary nucleic acid sequence of any thereof, said DNA sequence encoding a polypeptide or fragment thereof, involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

10. The isolated DNA sequence of claim 9, wherein said nucleic acid sequence comprises at least 40% homology with SEQ ID NO: 1A, SEQ ID NO: 2A, or a complementary nucleic acid sequence of either thereof.

11. The isolated DNA sequence of claim 9, wherein said nucleic acid sequence comprises at least 60% homology with SEQ ID NO: 1A, SEQ ID NO: 2A, or a complementary nucleic acid sequence of either thereof.

12. The isolated DNA sequence of claim 11, wherein said polypeptide or fragment thereof has alcohol acyl transferase activity.

13. The isolated DNA sequence of claim 9, wherein said amino acid sequence comprises SEQ ID NO: 3A or a fragment thereof, and said polypeptide or fragment thereof has aminotransferase activity.

14. The isolated DNA sequence of claim 9, wherein said amino acid sequence comprises SEQ ID NO: 4A or a fragment thereof, and said polypeptide or fragment thereof has thiolase activity.

15. The isolated DNA sequence of claim 9, wherein said amino acid sequence comprises SEQ ID NO: 5A or a fragment thereof, and said polypeptide or fragment thereof has pyruvate decarboxylase activity.

16. The isolated DNA sequence of claim 9, wherein said amino acid sequence is selected

from the group consisting of SEQ ID NO: 6A, SEQ ID NO: 7A, SEQ ID NO: 8A, SEQ ID NO: 9A, SEQ ID NO: 10A, SEQ ID NO: 11A, SEQ ID NO: 12A, SEQ ID NO: 13A and a fragment of any thereof, and said polypeptide or fragment thereof has alcohol dehydrogenase activity.

17. The isolated DNA sequence of claim 9, wherein said nucleic acid sequence comprises at least 65% homology with SEQ ID NO: 7A, SEQ ID NO: 8A, SEQ ID NO: 9A or SEQ ID NO: 10A, or a complementary nucleic acid sequence of any thereof.

18. The isolated DNA sequence according to claim 9, wherein said nucleic acid sequence comprises at least 30% homology with SEQ ID NO: 12A, SEQ ID NO: 13A, or a complementary nucleic acid sequence of either thereof.

19. A purified and isolated polypeptide or fragment thereof, involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, said purified and isolated polypeptide comprising:

an amino acid sequence selected from the group consisting of: SEQ ID NO: 1B, SEQ ID NO: 2B, SEQ ID NO: 3B, SEQ ID NO: 4B, SEQ ID NO: 5B, SEQ ID NO: 6B, an amino acid sequence having at least 30% homology with SEQ ID NO: 1B, amino acid sequence having at least 40% homology with SEQ ID NO: 2B, an amino acid sequence having at least 80% homology with SEQ ID NO: 3B, an amino acid sequence having at least 90% homology with SEQ ID NO: 4B, an amino acid sequence having at least 90% homology with SEQ ID NO: 5B, an amino acid sequence having at least 75% homology with SEQ ID NO: 6B, and a fragment of any thereof,

said polypeptide or fragment thereof being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

20. The purified and isolated polypeptide or fragment thereof, of claim 19, wherein said amino acid sequence comprises at least 50% homology with SEQ ID NO: 1B, SEQ ID NO: 2B, or fragment of either thereof.

21. The purified and isolated polypeptide or fragment thereof, according to claims 19 or 20, wherein said amino acid sequence comprises at least 70% homology with SEQ ID NO: 1B, SEQ ID NO: 2B, or fragment of either thereof.

22. The isolated DNA sequence encoding a polypeptide, of claim 21, wherein said polypeptide or fragment thereof, has alcohol acyl transferase activity.

23. The isolated DNA sequence encoding a polypeptide, of claim 19, wherein said polypeptide or fragment thereof comprises SEQ ID NO: 3B and has aminotransferase activity.

24. The isolated DNA sequence encoding a polypeptide, of claim 19, wherein said polypeptide or fragment thereof comprises SEQ ID NO: 4B and has thiolase activity.

25. The isolated DNA sequence encoding a polypeptide, of claim 19, wherein said polypeptide or fragment thereof comprises SEQ ID NO: 5B and has pyruvate decarboxylase activity.

26. The isolated DNA sequence encoding a polypeptide, of claim 19, wherein said polypeptide or fragment thereof comprises SEQ ID NO: 6B and has alcohol dehydrogenase activity.

27. A recombinant expression vector comprising:
a coding sequence operably linked to a promoter sequence and capable of directing expression of said coding sequence in a host cell of said vector, said coding sequence comprising the DNA sequence according to any one of claims 1 to 18; and
a transcription termination sequence.

28. A replicative cloning vector comprising:
the isolated DNA sequence of any one of claims 1 to 18; and

a replicon operative in a host cell for said vector.

29. A method for regulating aliphatic and/or aromatic ester formation in fruit, comprising inserting one or more copies of one or more DNA sequences according to claims 1 to 18 into a genome of a fruit-producing plant.

30. The method according to claim 29, wherein said plant is selected from the group consisting of strawberry, citrus, banana, apple, pear, melon, tomato, sweet pepper, peach and mango.

31. A plant and propagating material thereof comprising a genome including the vector according to claims 27 or 28.

32. A genetically modified strawberry or lemon plant and propagating material derived therefrom, comprising a genome having an expression vector for over-expression or down-regulation of an endogenous strawberry or lemon plant gene counterpart of the DNA sequences of claims 1 to 18.

33. A method of producing aromatic and/or aliphatic esters in a microorganism, plant cell, or plant, comprising:
inserting one or more copies of the DNA sequences of claims 1 to 18; and
feeding an alcohol CoA and an acyl-CoA to the microorganism, plant cell or plant.

34. A purified and isolated polypeptide, or fragment thereof involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, said purified and isolated polypeptide comprising:

an amino acid sequence selected from the group consisting of: SEQ ID NO: 7B, or a fragment thereof, SEQ ID NO: 8B, or a fragment thereof, SEQ ID NO: 9B, or a fragment thereof, SEQ ID NO: 10B, or a fragment thereof, an amino acid sequence having at least 55%

homology with a 326 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 7B, an amino acid sequence having at least 75% homology with a 278 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 8B, an amino acid sequence having at least 65% homology with a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 9B, and an amino acid sequence having at least 80% homology with a 188 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 10B, SEQ ID NO: 11B, an amino acid sequence having at least 75% homology with a 181 aa fragment from the C-terminal end of the coding sequence of SEQ ID NO: 11B, SEQ ID NO: 12B, SEQ ID NO: 13B, an amino acid sequence of a 176 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 12B, an amino acid sequence having at least 35% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 13B, SEQ ID NO: 15B, an amino acid sequence having at least 41% homology with SEQ ID NO: 15B, and a fragment of any thereof,

said polypeptide or fragment thereof having alcohol dehydrogenase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

35. An isolated DNA sequence encoding a polypeptide, or fragment thereof, said DNA sequence comprising:

an amino acid sequence selected from the group consisting of: SEQ ID NO: 7B, an amino acid sequence having at least 55% homology with the amino acid sequence of a 326 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 7B, SEQ ID NO: 8B, an amino acid sequence having at least 75% homology with the amino acid sequence of a 278 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 8B, SEQ ID NO: 9B, an amino acid sequence having at least 65% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 9B, SEQ ID NO: 10B, an amino acid sequence having at least 80% homology with the amino acid sequence of a 188 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 10B, SEQ ID NO: 11B, an amino acid sequence

having at least 75% homology with the amino acid sequence of a 181 aa fragment from the 3' end of SEQ ID NO: 11B, SEQ ID NO: 12B, SEQ ID NO: 13B, an amino acid sequence having at least 55% homology with a 176 aa fragment from the C terminal end of the coding sequence of SEQ ID NO:12B, and an amino acid sequence having at least 35% homology with a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 13B, SEQ ID NO: 15B, an amino acid sequence having at least 41% homology with the amino acid sequence SEQ ID NO:15B and a fragment of any thereof, said polypeptide or fragment thereof having alcohol dehydrogenase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

36. A method for regulating aliphatic and/or aromatic ester formation in fruit comprising inserting into the genome of a fruit-producing plant one or more copies of one or more the DNA sequences as defined in any of claims 1-18.

37. A genetically modified strawberry or lemon plant and propagating material derived therefrom which has a genome comprising an expression vector for overexpression or downregulation of an endogenous strawberry or lemon plant gene counterpart of any of the DNA sequences as defined in claims 1 -18.

38. A method for producing aromatic and/or aliphatic esters in a microorganism, plant cell or plant, comprising:
inserting into the genome of the microorganism or plant one or more copies of DNA sequences as defined in any of claims 1 to 18, and one or more copies of DNA sequences as defined in any of claims 19-26; and
feeding aldehydes and acyl-CoA to the microorganism, plant cell or plant.

39. A method for producing aromatic and/or aliphatic esters in a microorganism, plant cell or plant, comprising:
inserting into the genome of the microorganism or plant one or more copies of DNA sequences

as defined in any of claims 1 to 18, and one or more copies of DNA sequences as defined in any of claims 19-26; and

feeding alpha-keto acids and acyl-CoA to the microorganism, plant cell or plant.

40. The method for producing aromatic and/or aliphatic esters in a microorganism, plant cell, or plant according to claim 39, further comprising feeding fatty acids to the microorganism, plant cell or plant.

41. An isolated DNA sequence according to any of claims 1 to 18 obtainable by cloning using as a primer a nucleotide sequence encoding the amino acid sequence

- as set forth in SEQ ID NO: 14B, or
- LeuXXXTyrProXXGlyArg, or
- ProSerArgValXXValThrXPheLeuXLysX Leu lie

wherein X refers to any amino acid residue.

42. An antibody capable of specifically binding one or more polypeptides as claimed in any of claims 19-26.

43. A diagnostic kit for screening fruit with specific reference to volatile aliphatic and/or aromatic ester compounds comprising:

- a) one or more polypeptide as claimed in any of claims 19-26 or
- b) one or more DNA sequences as claimed in any of claims 1-18, or
- c) one or more antibodies as defined in claim 42.

44. A method for screening fruit comprising:
 screening fruit for quality trait attributes, such as flavor, fragrance, aroma, scent, texture, shape;
 distinguishing between cultivars/varieties of fruits based on their volatile ester profiles; or
 monitoring harvest time of fruit(s) for quality trait attributes, or
 monitoring post-harvest fruit(s) for quality trait attributes, or

monitoring shelf-life of fruits, or

monitoring timing of application of pesticides based on volatile ester profiles, or

monitoring fruit resistance capacity based on their volatile ester profiles, which comprises using the diagnostic kit as claimed in claim 43.